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DEVELOPMENT OF MICROCOMPUTER METHODS FOR ANALYSIS AND
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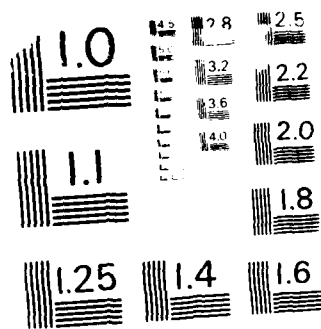
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Development of Microcomputer Methods for
Analysis and Simulation of Clinical
Pharmacokinetic Data Relevant to New
Drug Development

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Annual Report

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Duke University
Division of Clinical Pharmacology
Durham, North Carolina 27710

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The research proposed under this contract is a feasibility study in the development of applications of new microcomputer graphics technology to the analysis and interpretation of clinical pharmacological data. This involves continuing development of comprehensive programs for analysis, interpretation, and simulation of pharmacokinetic data, dose-response kinetic data, and other data relevant to new drug development, for use with the Tektronix 4052 Microcomputer Graphics System. The combination of such modern analytical and		

illustrative methods in clinical pharmacology, based on new high-speed micro-computers and associated graphics, are thought to greatly reduce both cost and time involved in the overall process of clinical evaluation of new drugs in the U.S. Army Drug Development Program.

The work performed during the past twelve months of the contract includes the following:

1. Continuing development of a non-linear pharmacokinetic data fitting program to analyze drug data where non-linear processes are responsible for some of the drug disposition pathways.
2. Development of a pharmacokinetic and pharmacodynamic data simulation program to simulate both time courses of drug concentrations and pharmacologic effect.
3. Continuing development of a statistical program package applicable to problems in clinical pharmacology, involving both parametric and non-parametric tests.
4. Development of a pharmacokinetic simulation program based on differential equations, intended to simulate time courses of concentrations of drugs exhibiting non-linear kinetics.
5. Initial development of a quantal (probit) dose-response analysis program.
6. Special analysis of data from WRAIR, one on a comparative bioavailability study of mefloquine, the other on methemoglobinemia induced by primaquine and related compounds.

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SUMMARY

The research proposed under this contract is a feasibility study in the development of applications of new microcomputer graphics technology to the analysis and interpretation of clinical pharmacological data. This involves continuing development of comprehensive programs for analysis, interpretation, and simulation of pharmacokinetic data, dose-response kinetic data, and other data relevant to new drug development, for use with the Tektronix 4052 Microcomputer Graphics System. The combination of such modern analytical and illustrative methods in clinical pharmacology, based on new high-speed microcomputers and associated graphics, are thought to greatly reduce both cost and time involved in the overall process of clinical evaluation of new drugs in the U.S. Army Drug Development Program.

The work performed during the past twelve months of the contract includes the following:

1. Further development of a non-linear pharmacokinetic data fitting package to analyze drug data in systems where some non-linear processes are responsible for transport or metabolism of the drug (e.g. Michaelis-Menten kinetics). This is a continuation of work begun last year. Some of the causes for the disappointing slowness of the original programs have been identified, as well as some limits on the degree of improvement which is achievable.

2. Creation of a pharmacokinetic and pharmacodynamic data simulation program which enables the user to simulate the time course of drug concentration and effect. This program extends the capabilities of our previously developed pharmacokinetic simulator to allow the examination of effect vs time relationships for various drug dosing regimens. The user can now have graphs of the predicted concentration and/or effect data resulting from any type of dosing regimen, with the same ease of use and flexibility available from the original pharmacokinetic simulation program.

3. Additional work on statistical programs for clinical pharmacology. One of our main efforts in the statistical package was the implementation of several multiple-comparison tests. These tests are particularly useful in simultaneous comparative studies of several drugs, allowing comparison of all possible sets of competing treatments in order to quickly isolate the most promising for further study. This enables more efficient use of time and money in testing new experimental courses of treatment, reducing the development cost of new drugs.

4. Development of a pharmacokinetic simulation program based on differential equations. This program is intended to be used as a companion to the non-linear pharmacokinetic data analysis package, allowing the prediction of time courses of drug concentrations due to various dosing regimens in systems characterized by rate-limited processes of drug transport and/or metabolism. It provides functions similar to those available in our linear pharmacokinetic simulator, which was previously developed.

5. Work on a probit (quantal) dose-response analysis program. This type of pharmacodynamic analysis is commonly used in studies involving responses of a discrete, categorical nature, rather than the continuous effect data which is dealt with by our previous dose-response analysis program. The two techniques can overlap somewhat if the discrete responses are converted to percentages of some total available response. If this is done, the two programs produce very similar results for the ED-50, or median effective dose, but the bounds placed on this estimate are sometimes quite different. This discrepancy between the two methods is a matter we intend to examine further during the coming year.

6. Special analysis of data from the Walter Reed Army Institute of Research. Two requests were received for assistance with data analysis in connection with drug development experiments being performed at WRAIR. One was from LTC Charles Pamplin, asking that we analyze the results from a comparative bioavailability study of two formulations of mefloquine which was performed on 12 normal subjects using a cross-over experimental design. This experiment was complicated by the fact that one of the subjects was mistakenly given the two treatments in the wrong order, resulting in an unbalanced study. We have managed to successfully overcome this difficulty in the analysis, and are currently preparing a final report on the results.

The other special request came from CPT John Anders, who has performed experiments on the degree of methemoglobinemia in dogs given primaquine and several new antimalarial compounds under development. One of his questions involved the pooling of results from the primaquine-treated groups in two different experiments, in order to obtain a larger sample and thus greater statistical power. The analysis of his data also involved the use of some of the multiple comparison methods we have developed this year, which are discussed in this report.

BACKGROUND

The process of drug development has been both complicated and facilitated by the trend toward early application of the methods of clinical pharmacokinetics. Current practice and existing and proposed new Food and Drug Administration Regulations demand sophisticated evaluation of drug bioavailability and descriptive pharmacokinetics in Phase I clinical studies. This requires development of assay methods for new drugs and their metabolites and methods for evaluation of concentration vs time data to obtain relevant parameters to characterize drug behavior. Of similar importance is knowledge of characteristics of relationships between dose or concentrations and pharmacological response. The information thus obtained from pharmacokinetic and pharmacodynamic studies relates directly to the optimal design of dosing schedules of new drugs in man, including individualization of therapy due to disease processes or other factors which may affect drug behavior. In this regard the eventual course of development through Phase II and Phase III clinical studies is rendered less empirical and ultimately more efficient in both time and cost, by minimizing the use of scarce resources.

Modern computer technology has greatly enhanced the capability of these methods and made possible their applications to clinical pharmacokinetics. A number of available computer programs have been frequently employed for this purpose. Those which have been developed for use specifically in clinical pharmacokinetics are based on compartmental methods of analysis and yield estimates of parameters associated with preselected compartmental models. While useful and informative, they lack the ease of use and cognitive appeal of direct graphic simulations and graphics-assisted data analysis. It is in the interest of developing general purpose programs with the advantages of computer graphics that the present contract was initially pursued.

Previous experience with the Tektronix 4051 and 4052 Microcomputer Graphics Systems indicated that this was an especially suitable microcomputer system for our purposes. While similar systems are now available from a variety of sources, it is in the interest of conformity with existing systems in the U.S. Army Drug Development Program at the Walter Reed Army Institute of Research that the Tektronix System has been employed. The present Tektronix 4052 System in our laboratory and for which the programs to be described were developed is identical to that now in use at Walter Reed. A data communications interface has been installed to facilitate direct transmission of programs and data between these two facilities.

PROGRESS

A. Non-linear Pharmacokinetic Data Analysis Package Using Differential Equations.

This program package, on which work was begun last year, is intended to be used for fitting data to a model involving some non-linear kinetic processes. Such systems cannot be analyzed by model-independent methods such as our linear pharmacokinetics program package, developed earlier under this contract. Because it is important to be able to characterize these data in cases where a new drug shows evidence of some rate-limited or time-dependent kinetic behavior, this package has been designed to allow the fitting of a model described by a system of differential equations, which need not be restricted to the linear, first-order models used for simpler pharmacokinetic analysis. In developing this package, we have tried to retain many of the "user-friendly" aspects of our other pharmacokinetics package, including extensive graphic interaction for verification of data values and initial estimates of the model parameters. The detailed statistical analysis of the results of the fitting procedure has also been retained, although not as much information can be derived by the program with regard to clearance terms, volumes of distribution, or half-times, since the program has no knowledge of the meaning of the model parameters other than time and concentration in the sampling compartment.

The principal reason for our dissatisfaction with the program last year was the fact that complete analysis of one set of data took much longer than a similar problem would using the linear pharmacokinetic analysis package. A number of possible solutions for this difficulty were tried, specifically including testing several different algorithms for the numerical integration of the system of differential equations which describes the model. These attempts were only somewhat successful, for reasons which became apparent after trying many of the different methods. One of the main difficulties in numerically solving a differential equation system is the choice of an appropriate step-size to use in moving from one observed data point to the next. Sophisticated methods exist for adjusting the step-size so as to maintain the desired level of accuracy without taking more steps, and time, than necessary. However, due to the unequal spacing frequently found between successive observations in pharmacological studies, automatic step-size adjustment becomes impractical, because the solution must be restarted for each new interval, and the resulting increases in overhead and bookkeeping in the program waste most of the time saved by using the more elaborate algorithm. A further cause for the slowness of the program is the fact that the numerical integration of the model with respect to time must be followed by a stage of numerical differentiation with respect to all the other parameters. This is in marked contrast to the linear pharmacokinetics program, which uses analytic derivatives which have been written

into the code. We feel that this may be the most time-consuming step in the solution process, and that further attempts to reduce the running time would not be productive enough to justify the effort. Therefore, to simplify the program, the complex predictor-corrector method which was being used has been replaced by a fourth-order Runge-Kutta integration technique, along with a simple means of determining step size. These changes reduce the size of the program significantly, and should also make it easier to maintain and modify in the future.

B. Pharmacokinetic and Pharmacodynamic Simulation Program.

This program is an extension of our earlier pharmacokinetic simulator to add the capability of predicting the time course of effect of a drug, based on the predicted concentration and on a concentration-effect relation like the one used in our program for pharmacodynamic analysis. The resulting program allows the user to request that the predicted effect be plotted against time alone, or with the predicted concentrations as well. If concentrations are to be graphed, they may be shown on linear or logarithmic scales, as in the original program. The effect axis is always linear and will be automatically scaled by the program, but the user may override the choice of endpoints and tic intervals if desired, another feature which has been retained from the earlier program. A new capability with this version, which is most useful when drawing graphs using the Tektronix plotter, is the pause feature, which stops the pen after drawing the axes and labels, and again after the concentration curve and before the effect curve, allowing the changing of pen colors for different sections of the plot. In addition to these changes, some other improvements have been added to reduce the drawing time of the program, resulting in a more uniform line width on the paper, which is less likely to smear. This program should prove most useful in designing drug dosage regimens on the basis of some desired range of effectiveness, such as to maintain a minimum effective level, or to avoid a level of effect which is associated with negative drug reactions.

C. Statistical Program Package for Clinical Pharmacology.

Most of the development of the statistical program package this year has been concentrated on the problem of performing multiple comparisons among several groups of data without inflating the apparent significance of the results. This problem can occur frequently in pharmacology research in situations where a pilot experiment is performed on a number of new drugs or methods of treatment in order to determine which is the most promising for further investigation. A similar situation arises when an established standard compound or formulation is to be compared against several new ones, to see if there are differences in quality. The difficulty with this type of comparison is that the various subsets of the data that are to be contrasted are not generally independent, and this

can lead to stated probability levels which are much more significant than the actual test result ("false positives"). The techniques used for avoiding this problem all involve making the individual comparisons at more stringent probability levels, so that a statement can be made about all the tests simultaneously at an overall significance level of 0.05 or 0.01 or whatever level is desired. Procedures for doing this have now been added to our non-parametric analysis of variance program, the Kruskal-Wallis test for one-way classifications, and the Friedman test for data in a two-way layout. Additionally, we have written a program for the parametric one-way analysis of variance with multiple comparisons, using the Bonferroni method for adjusting the significance levels. Two other tests are being investigated for the parametric analysis of variance program: Dunnett's test for comparing all other treatments vs a control or standard; and Duncan's for all possible comparisons. Because these methods currently require the use of tabular values in order to apply the tests, we are hoping to find a way to have the necessary critical values computed within the program, making it more useful. One other development for the statistical program package has been the beginning of a method for analyzing data from unbalanced cross-over design studies. This problem was brought to our attention in connection with the analysis of mefloquine bioavailability data requested by LTC Charles Pamplin. Because a cross-over design may easily be upset by accident, or by uncooperative subjects, the ability to analyze data resulting from an unbalanced experiment is most important in order to learn as much as possible from the completed sections of the study. We intend to do further development work on this program in the coming year.

D. Non-linear Pharmacokinetic Simulation Program Using Differential Equations.

This program complements the capabilities of the non-linear pharmacokinetic data fitting package by allowing the prediction of concentration vs time curves for the same kinds of kinetic models defined in terms of sets of differential equations. In this, it may be even more valuable than our linear kinetic simulator in helping people to visualize the effects of changes in dosage regimens, since the non-linear models are more difficult for most people to grasp intuitively. As much as possible, we have tried to make this program as easy to use as the corresponding linear one, with the same options for such features as titles, axis ranges, and log/linear scaling. In addition, this program also has the pre-computation of the entire time-course before drawing the graph in order to reduce the drawing time and produce a more uniform looking graph. The only significant feature not yet available in the non-linear simulation program is the ability to make effect predictions based on the sample compartment concentration. This section has been left out of the current version of the program not because of any problem with implementation, but instead because we do not know of any good source of data concerning concentration-effect relations in non-linear kinetic models. Without such a source, this portion of the program cannot be properly tested.

E. Quantal Dose-Response Analysis Program using Probits.

The purpose of this program is to allow the analysis of dose-response data in situations where the measured response is discrete or categorical, rather than the continuously variable responses dealt with by our earlier pharmacodynamic analysis program. Categorical data can arise in many different circumstances, including experiments where the response can only be roughly measured and those in which the response is an event which has an all-or-nothing character, such as death in an LD-50 study. The method used for analyzing such data involves converting the percentage responding at each dose level into probability units, or probits, and then performing a linear regression of probit response vs log dosage. This has the effect of fitting a cumulative normal probability curve to the dose-response data. Because of the nature of this curve, the initial fitting is not usually sufficiently accurate to fully describe the data. To correct this, weights are assigned to each data point based on its position on the initial regression line, and a new weighted regression is computed. This process is then repeated, with new weights drawn from the new regression line. This cycle is repeated until the values at two successive stages of the iteration are judged sufficiently close. The program runs fairly quickly, with convergence to a final answer typically occurring after four to eight cycles of this re-weighted regression. The resulting line corresponds to a normal probability curve, with the mean representing the ED-50 and the standard deviation giving a measure of the sensitivity of the effect to changes in the dose. During comparison of this program to the logistic pharmacodynamic data analysis program, we obtained very consistently comparable estimates of the ED-50.

F. Special Data Analysis Requests from Walter Reed.

Two major analysis projects were performed this year at the request of researchers from the Walter Reed Army Institute of Research. One, requested by LTC Charles Pamplin, involved the treatment of data from a cross-over design comparative bioavailability study of two tablet formulations of mefloquine. This was somewhat similar to an earlier analysis of data which we performed in the previous contract year. The second analysis project, for CPT John Anders, was an examination of measured methemoglobinemia in dogs, caused by dosing with primaquine or with new experimental antimalarial compounds. Details on both of these analyses follow.

LTC Pamplin asked us to analyze the data from a study performed by Bio-Med, Inc. for WRAIR on the comparative bioavailability of two tablet formulations of mefloquine, lot E555 prepared by Lafayette Pharmacal and a second prepared by Hoffman-LaRoche. The study was intended to be a balanced cross-over experiment, with 12 normal subjects receiving both preparations, with half the subjects to be given the tablets in one order

and the other half in the reverse order. Since it was assumed that the volume of distribution and rate of elimination of mefloquine in a given individual would be independent of the formulation given, our analysis focused on possible differences in the rate of absorption and in the fraction of the total dose which was actually available. This analysis was complicated by two factors. First, due to the withdrawal of one of the original test subjects during the study, a replacement was added, but he was inadvertently given the formulations in the reverse order of the individual who left the study. This resulted in an unbalanced cross-over design, which complicated the statistical analysis. Second, due to the long half-life of mefloquine, a significant amount of residual drug from the first dose was still present in the subjects when the second formulation was administered. This fact complicated the pharmacokinetic analysis. Nevertheless, we were able to perform a detailed analysis of the data, as summarized below.

The data regarding dosing order, times of administration, body weight, measured concentrations during the study, etc. were transmitted through telephone lines from WRAIR to Duke, using the data communications interface of our Tektronix 4052. After receiving this set of data, we performed some extensive reformatting to enable the subsequent pharmacokinetic analysis steps to access all the data. Two principal steps were taken in the kinetic analysis of the concentration vs time data. First, the area under the curve was calculated using a combination of the trapezoidal rule and extrapolation beyond the last data point based on the terminal exponential curve. For each subject, this calculation was performed in such a way as to force the exponential slopes after the first and second doses to be identical. In addition, the area under the second dosing interval curve was corrected for the carry-over of the exponential tail from the first dosing interval. Second, a non-linear regression analysis was performed, fitting the concentration data to a model which allowed different absorption rates and fractional availabilities for the first and second doses, but kept the volume of distribution and elimination rate terms identical during both dosing intervals. The resulting collection of pharmacokinetic parameters was then statistically analyzed, using an analysis of variance model for an unbalanced cross-over design. The results of this analysis showed a consistent and statistically highly significant pattern of differences between the two formulations. The data conclusively showed that the Hoffman-LaRoche preparation was more rapidly and more completely absorbed. In addition, this project showed that useful data can be obtained from bioavailability studies in which there is some interference between successive stages, and from imperfect experimental designs. Preliminary results from this study have already been sent to WRAIR, and a final report is currently being prepared for submission.

CPT Anders requested our assistance in analyzing data from two experiments which dealt with methemoglobinemia in dogs caused by primaquine or by one of several new experimental antimalarials. In the first experiment, primaquine was compared with WR238605, WR225448, and WR242511. In the second, primaquine was compared with WR6026. In both studies, molar equivalent doses of the drugs were given. The objective of this analysis was to compare all the drugs to determine if any statistically significant differences in methemoglobinemic potential existed. Prior to making such a comparison, however, we had to verify that the primaquine data in the first experiment was comparable to that in the second, so that pooling of these two sets of data would be legitimate.

Data from a total of 19 dogs were presented for analysis. The desired comparison was to be on the basis of percent methemoglobinemia (Mhb) caused by administration of one of the antimalarial compounds in four daily doses. Because of the significantly non-zero baseline (pre-dose) values of methemoglobin in the dogs, corrections had to be made in order to compare only the effect of the drug. This was achieved by fitting each dog's Mhb vs time data to a model based on a one-compartment kinetic system with first-order input, modified to have a non-zero baseline as one of the model parameters. The results of this analysis were then treated with both parametric and non-parametric analysis of variance procedures with multiple comparison testing, as described earlier in this report. The parameters tested were baseline Mhb, rate of increase during dosing, rate of decrease after dosing, theoretical initial Mhb level, and total area under the curve above baseline. Preliminary comparison of the primaquine data from the first and second experiments showed no significant differences, so these two groups were pooled for all subsequent analyses. The results showed no pattern of differences between groups for baseline, nor for rate of Mhb increase or decrease. Statistically significant differences were found in the theoretical initial Mhb level and in the area under the curve, with WR242511 being significantly higher than any of the other groups. No other significant differences were detected by this procedure. In particular, the new compound which resulted in the lowest Mhb levels, WR6026, was not significantly higher than primaquine, which was lowest, under direct comparison. A detailed report on the results of this analysis has been submitted, and a copy is attached as an appendix to this report.

Technical Report

Statistical Comparison of Methemoglobin-Producing
Potential of Five 8-Aminoquinoline Antimalarial Drugs

September 21, 1983

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INTRODUCTION

CPT John Anders of the Walter Reed Army Institute of Research presented data from two experiments, involving a total of 19 subjects (male beagles), concerning the methemoglobinogenic potential of five different 8-aminoquinoline antimalarial drugs. The purpose of these experiments was to compare primaquine, WR2975, with four new compounds, WR238605, WR225448, WR242511, and WR6026, with respect to the degree and duration of drug-induced methemoglobinemia. The analysis of the data was complicated by the fact that there were significant levels of baseline methemoglobin which had to be taken into account in order to compare the different drugs. An additional concern dealt with the fact that control groups in each of the two experiments were given primaquine, but it was not known whether these two groups could be pooled together. These problems were solved, using nonlinear regression analysis to describe the production and elimination of methemoglobin in each dog, and using parametric and nonparametric statistical methods for comparing multiple groups. The results showed some very important differences among the drugs, and indicated that two of them, WR238605 and WR6026, would be the most promising for further investigation.

METHODS

The initial problem faced in analyzing this data was the choice of an appropriate model to describe the time course of methemoglobin formation and elimination in the individual dogs. Because no information was available on the drug concentrations, it was not possible to perform a simultaneous pharmacokinetic and pharmacodynamic analysis to describe the time course of drug concentration and the concentration-effect relationship between drug concentration and methemoglobin level. This forced the choice of a simpler pharmacokinetic model to fit to the data. In addition, the model had to be modified to take into account the baseline levels of methemoglobin, which were quite high in some cases, and which varied widely among different animals. The model finally chosen was a single-compartment multiple-dose kinetic system, with first-order input and output, with an additional constant term to correct for baseline. The parameters of this model were baseline, C_0 , k_a , and k_{el} . The baseline term reflects the initial pre-dose level of methemoglobin, based on one week of baseline data, as well as the limiting terminal concentrations at the end of the experiment. C_0 is usually interpreted as the theoretical concentration at time 0, if instantaneous drug absorption were to take place. In this case, C_0 represents the initial percentage methemoglobinemia which would result if both drug absorption and drug-caused methemoglobin production took place instantly. k_a is normally a measure of drug absorption rate; in this model it probably reflects both the absorption rate of the drug and the rate of formation of methemoglobin. k_{el} is usually taken to represent the drug elimination rate; in this case it may be a measure of elimination of drug or methemoglobin, or a combination of both processes. Using this model, nonlinear regression analysis was performed on each individual animal's methemoglobin measurements. The resulting fitted curves were examined to see if the model adequately described the data. The results were surprisingly good, showing no pattern of deviations that would suggest a more complex model was needed. The parameters describing these individual curves were then tabulated and summarized to prepare for the statistical comparisons to be performed. An additional descriptive parameter was computed, the area under the curve (AUC). This is the integral of the level of methemoglobin due to drug across time, after correcting for the baseline values. AUC provides a measure of the total methemoglobinogenic impact of a particular drug, since increases in level or duration of effect will cause increases in AUC.

After computing the best-fitted curves for each set of data and collecting the resulting parameters, statistical testing was performed, using both parametric and nonparametric techniques. The primaquine control groups from the first and second experiments were compared and found to be well-matched, so these groups were pooled together for all subsequent comparisons against the other drugs. The data were classified into groups based on which drug had been given, and these groups were then

tested for any significant difference using the classical one-way analysis of variance and its nonparametric equivalent, the Kruskal-Wallis test. These procedures for detection of overall differences were augmented by multiple comparison procedures to allow pair-wise testing of groups in order to isolate the areas where important differences were found. These methods are designed to control the likelihood of a so-called Type I error, that is, concluding that a significant difference exists when the actual cause is random variation. In this study, the possibility of a Type II error, failure to detect a real difference, was also a great concern. Therefore, additional tests were performed in order to assure that those groups which were declared "not significantly different" were truly comparable.

RESULTS

The individual parameters from the curve-fitting procedure are listed in Table 1, along with groups means and standard deviations and overall means and standard deviations. The results of the statistical tests by parametric and nonparametric methods were almost identical, with both approaches finding significant effects in C_0 and AUC, but no systematic differences in any of the other parameters. Baseline methemoglobin levels seemed to be a random characteristic of each dog, and k_a and k_{el} showed no patterns of being faster or slower for any particular drug. The multiple comparison procedures showed that WR242511 caused significantly higher methemoglobin levels, as measured by C_0 and AUC, than primaquine, WR238605, and WR6026. There was also a smaller significant difference between WR225448 and primaquine. When individual tests were performed to maximize the power of detecting small differences, no statistically significant differences were found between primaquine and WR238605, nor between primaquine and WR6026. These two compounds would appear to be the most promising for further study, since when given in equimolar amounts as primaquine, they cause no significantly higher levels of methemoglobinemia.

Table 1: Summary of parameters of fitted curves describing the time courses of methemoglobin levels in individual animals.

	Baseline	C ₀	k _a	k _{el}	AUC
WR2975					
2A30	0.4321	1.432	0.07981	0.008743	163.8
2A20	1.0458	1.320	0.04248	0.009592	137.6
2A21	4.5192	2.741	0.05370	0.006564	417.6
2D93	6.3253	3.030	0.02039	0.020385	148.6
2D95	0.4711	0.958	0.07285	0.008483	112.9
2D96	2.6197	1.903	0.13725	0.002596	733.1
Mean	2.5689	1.897	0.06775	0.009394	285.6
S.D.	2.4167	0.828	0.04020	0.005938	246.2
WR238605					
2A23	0.3714	10.824	0.01487	0.006170	1754.3
2A26	0.3487	5.565	0.00690	0.006896	806.9
2A31	1.9596	2.993	0.02122	0.001357	2205.6
Mean	0.8932	6.460	0.01433	0.004808	1588.9
S.D.	0.9236	3.992	0.00718	0.003010	713.9
WR225448					
2A25	2.2463	7.470	0.05045	0.001673	4465.5
2A22	1.4132	13.387	0.01012	0.005126	2611.8
2A27	0.2983	5.513	0.01764	0.003728	1478.8
Mean	1.3193	8.790	0.02607	0.003509	2852.0
S.D.	0.9774	4.100	0.02145	0.001737	1507.7
WR242511					
2A28	1.2719	15.193	0.06037	0.004330	3508.5
2A24	0.9122	17.584	0.02744	0.004116	4272.3
2A29	0.9842	18.623	0.01665	0.006048	3079.0
Mean	1.0561	17.133	0.03482	0.004831	3619.9
S.D.	0.1903	1.759	0.02278	0.001059	604.4
WR6026					
2D94	4.2800	5.042	0.07176	0.004314	1168.9
2D97	4.1263	6.792	0.07178	0.004271	1590.2
2D98	0.3562	7.188	0.03571	0.007487	960.0
2D99	4.2543	2.574	0.07952	0.004431	580.9
Mean	3.2542	5.399	0.06469	0.005126	1075.0
S.D.	1.9332	2.102	0.01966	0.001576	420.9
<hr/>					
Mean	2.0124	6.849	0.04689	0.006122	1589.3
S.D.	1.8269	5.642	0.03341	0.004143	1405.2

TECHNICAL REPORT

BIO-MED EXPERIMENT #10

COMPARATIVE BIOAVAILABILITY OF TWO TABLET FORMULATIONS OF MEFLOQUINE HYDROCHLORIDE

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Introduction

A series of oral formulations of mefloquine were produced during the development of this new antimalarial drug. The first to be used in Phase II therapeutic trials was a 250 mg (mefloquine-HCl) capsule manufactured by Lafayette Pharmacal. Results of these initial clinical trials suggested limited bioavailability of the capsule formulation. A subsequent 250 mg (mefloquine-HCl) tablet formulation, E443, also manufactured by Lafayette Pharmacal was more successful therapeutically.¹

A later 250 mg (mefloquine-HCl) tablet formulation, B512, was prepared by InterX with in vitro dispersion superior to that of E443. Extended Phase I safety, tolerance and pharmacokinetic studies with the new formulation, B512, suggested that at doses in excess of 1000 mg, it was not as well tolerated as E443 had been in earlier studies. A comparative oral bioavailability study with B512 and E443 demonstrated that the former was more rapidly and possibly more completely absorbed than the latter.²

A new lot, E555, of 250 mg (mefloquine-HCl) tablets was prepared by Lafayette Pharmacal according to the same formulation as E443. A tablet containing 250 mg mefloquine base (approximately 275 mg mefloquine-HCl) was also prepared by Hoffmann-LaRoche, Basel, Switzerland (HLR) for use in clinical trials supported by Hoffmann-LaRoche and under the auspices of the Steering Committee on the Chemotherapy of Malaria (CHEMAL) of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR). The present study was conducted to compare the oral bioavailability in normal healthy volunteers of the E555 and HLR tablet formulations.

Study Design and Methodology

This was to have been a standard randomized balanced cross-over study in which the safety, tolerance and oral bioavailability of a single 1000 mg (mefloquine-HCl) dose of the E555 formulation (4 tablets) would be compared with a single 1100 mg (mefloquine-HCl) dose of the HLR formulation (4 tablets). Both formulations were to be administered in random sequence to each of 12 volunteers in three groups of 4, with an interval of 28 days between the two doses. One of the participants (#263) failed to return for the second dose and was replaced by a 13th individual (#273). However, the replacement, #273, was given the two formulations in the opposite sequence assigned to the original participant, #263. Therefore, the resulting study was an unbalanced cross-over. While the data are still amenable to analysis there is a loss of power to detect significant differences because of this circumstance.

The sequence of administration to each of the volunteers is shown in Table 1, where A designates the HLR and B the E555 formulations. It should be noted that in this study the dose of mefloquine administered to each individual was not exactly the same for the two formulations. This was due to the fact that HLR was manufactured to contain 250 mg of mefloquine base and E555 to contain 250 mg of mefloquine-HCl. The administered dose was 4 tablets in both cases resulting in a total dose of 1100 mg for A and 1000 mg for B of mefloquine-HCl. These were the values employed in the subsequent data analysis.

Details of volunteer selection, drug administration and the blood sampling schedule for drug assays are described in the Final Clinical Report, Experiment Number 10, Bio-Med Inc. (January 9, 1979), a copy of which is appended (Appendix I). Whole blood concentrations of mefloquine were determined by a high pressure liquid chromatographic method reported previously.³

Compliance with the schedule of blood samples for drug assay was excellent for all of the 12 volunteers who completed the study. The exact sampling times and drug concentrations for each participant are presented in Appendix II.

Data Analysis

These data were evaluated in a number of different ways to compare the rate and relative extent of systemic absorption from the two formulations. Non-linear curve fitting to standard pharmacokinetic models* was performed in a Tektronix 4052 Graphics Computer System with programs developed in the Division of Experimental Therapeutics, Walter Reed Army Institute of Research and later modified in the Division of Clinical Pharmacology, Department of Medicine, Duke University. Statistical analyses were performed using the Statistical Analysis System (SAS) at the University of North Carolina.

A simple comparison of the peak concentrations of mefloquine (Table I) was made using the analysis of variance (ANOVA) for a cross-over design with terms in the model for treatment, subject and period effects. These peak concentrations were corrected according to the dose administered (A=100 mg, B=1000 mg) but not for body weight or estimated residual following the first dose.

A similar comparison of the areas under the concentration-time curve following each formulation was made. In this case the areas were calculated by the trapezoidal rule from time 0 to the final measured sample plus the extrapolated terminal portion of the curve based on a single exponential regression of the points on the curve beyond 96 hours. In addition, the area under the second concentration-time curve (Period 2) in each case was corrected by subtracting the extrapolated area under the first curve beyond the time of the second dose (i.e., residual area from Period 1). The plotted time concentration data and fitted terminal exponential regression in each case are shown in Appendix III.

From the areas thus estimated an apparent clearance term was calculated by dividing the area into the dose administered in each case:

$$Cl_{app} = \frac{D}{Area}$$

These clearance terms, shown in Table 3, were then compared by ANOVA for a cross-over design. Based on the assumption that the volume of distribution (V) and elimination rate (Kel) of mefloquine is the same regardless of the formulation in which it is administered, and the following relationship:

$$Cl_{app} = Kel \cdot V \cdot F$$

significant apparent differences in clearance between the two formulations are directly attributable to differences in fractional absorption (F).

Finally, all of the concentration-time data following administration of both formulations were fitted for each participant to a model which assumed that the volume of distribution and elimination rate constants for both formulations were the same but that the absorption rate constant and fractional absorption could vary independently. The parameters of this model, therefore, were:

- V = Volume of distribution
- k_{el} = Elimination rate constant
- k_{a1} = Absorption rate constant for B (i.e., E555)
- k_{a2} = Absorption rate constant for A (i.e., HLR)
- F_1 = Fractional absorption for B (E555)
- F_2 = Fractional absorption for A (HLR)

The clearance terms (see Table 4) thus obtained were similarly compared by ANOVA for a cross-over design, again to test indirectly the hypothesis that $F_1 = F_2$. In addition, the mean ratio of F_1/F_2 was calculated and its standard variance from unity tested by a one-sample t-test. The mean ratio of k_{a1}/k_{a2} was similarly evaluated. Plots of model-fitted curves are shown for each individual in Appendix IV.

Results

The corrected peak concentrations in each individual following formulation A (HLR) and B (E555) are shown in Table 1. The curves comparing these values (Appendix V) shows a significant difference among subjects (p=0.04) but no significant period effect (p=0.54). There was also a significant treatment difference in peak concentrations (p=0.02) favoring formulation A.

The clearance terms computed from areas obtained by the trapezoidal rule plus exponential extrapolation of terminal points were similarly compared. These corrected clearance terms for each formulation are shown in Table 3. The ANOVA comparing these values (Appendix VI) shows a significant difference among subjects ($p=0.0025$), no significant period effect ($p=0.9229$) and a significant difference between treatments ($p=0.0150$) favoring a higher fractional absorption for Formulation A.

The fitted curves (see Appendix IV) provided a set of parameter estimates for each participant including an elimination rate constant from which the half-life was calculated. These values are shown for each subject in Table 4. The mean half-life in these 10 volunteers was approximately 12 days which corresponds well with previously reported results.⁵ The clearance terms computed from the fitted curves are shown in Table 5. The ANOVA for these values (Appendix VII) similarly shows a significant difference among subjects ($p=0.0010$), no significant period effect ($p=0.4484$), and a significant difference between treatments ($p=0.0034$), again favoring a higher fractional absorption for formulation A.

The values for the absorption rate constants (k_a) estimated independently for each formulation and their ratio (formulation A/formulation B) are shown in Table 6. The mean ratio was 1.5589 and was significantly greater than 1.0 ($p=0.0009$).

The values for fractional absorption as a function of the volume of distribution (F_v) are shown in Table 7, based on the assumption that the volume of distribution for melphalan is the same regardless of the formulation in which it is administered; the ratio of fractional absorption of the two form-

ulations (A/B) can be obtained by dividing these terms. The mean ratio was 1.3735 and was significantly greater than 1.0 ($p=0.0012$).

Conclusions and Discussion

Analyses of the blood concentration-time data from this comparative oral bioavailability study, by a variety of statistical and pharmacokinetic models, all indicate that the HLR tablet formulation containing 250 mg of mefloquine base was more rapidly and completely absorbed than was the Lafayette Pharmacal E555 tablet formulation containing 250 mg of mefloquine-HCl. Corrections were made in these analyses for the difference in dose (500 mg versus 250 mg of the HCl salt), but not for differences in body weight of the individual participants. There is some controversy about whether or not such a correction should also be made, but in this case a correlation analysis of the parameter of interest (clearance) with body weight of the individual participants showed that no such correlation existed (coefficient correlation = 0.476, $p=0.1217$).

In a previous comparative bioavailability study (formulation E-43), which was also manufactured by Lafayette Pharmacal and was identical to the present E555, was compared with Interyx formulation E512. The latter was more rapidly and possibly more completely absorbed. Prior clinical observations suggested the more frequent occurrence of particularly CNS side effects with E43 than had been the case with E443. Since the HLR formulation also produced significantly higher peak blood concentrations of mefloquine with more rapid and complete absorption of the drug than does the E555 formulation, a higher than expected frequency of adverse experiences may be observed with the use of the HLR formulation.

The methods employed in this comparative oral bioavailability study demonstrate that it is not absolutely essential to allow sufficient time to elapse between doses to reach nondetectable blood concentrations of the drug. In the case of a drug like mefloquine, with a half-life greater than 10 days, this would clearly be impractical. It is nevertheless quite possible to compare the fractional absorption of two formulations.

TABLE 1

A = HLR tablets 250 mg mefloquine base

B = E555 tablets 250 mg mefloquine-HCl

<u>Volunteer #</u>	<u>Period 1</u>	<u>Period 2</u>
261	B	A
262	A	B
263	A	--
264	B	A
265	B	A
266	B	A
267	A	B
268	A	B
269	A	B
270	A	B
271	B	A
272	B	A
273	B	A

Note: There are five individuals with sequence AB and seven with BA. The cross-over is, therefore, unbalanced.

TABLE 2

Peak Concentration ($\mu\text{g/ml}$) of Mefloquine
(Corrected to 1000 mg Dose)

<u>Subject</u>	<u>FORMULATION</u>	
	<u>A</u>	<u>B</u>
261	0.737	0.383
262	0.750	0.802
264	1.229	0.628
265	1.054	0.928
266	0.811	0.611
267	1.211	1.025
268	0.636	0.498
269	0.623	0.678
270	0.629	0.403
271	0.932	0.873
272	0.838	0.312
273	0.957	0.615

TABLE 3

Total Body Clearance of Mefloquine

Dose Divided by Area Under Concentration-Time Curves by Trapezoidal Rule
and Extrapolation of Single Exponential Fit of Terminal Data
(beyond 96 hours)

<u>Subject</u>	<u>FORMULATION</u>	
	<u>A</u>	<u>B</u>
261	3.339	5.283
262	3.607	3.307
264	1.274	2.168
265	2.297	3.067
266	3.887	4.407
267	1.338	2.292
268	4.891	6.252
269	3.565	3.930
270	5.126	1.474
271	3.771	4.262
272	3.626	4.267
273	4.774	6.027

TABLE 4

<u>Subject</u>	<u>Kel</u>	<u>t₁ (days)</u>
261	0.001531	18.86
262	0.002065	13.99
264	0.001410	20.48
265	0.002403	12.02
266	0.002219	13.02
267	0.001539	18.77
268	0.002559	11.29
269	0.002192	13.18
270	0.005081	5.68
271	0.003021	9.00
272	0.003004	9.61
273	0.003768	<u>7.66</u>
Mean		11.96

TABLE 5

Total Body Clearance (ml/min)
 Obtained from Fitted Regression Curves:
 Bicexponential Model

Subject	Formulation	
	A	B
261	3.158	4.582
262	2.940	3.653
264	1.558	2.497
265	2.633	3.495
266	3.761	3.916
267	1.735	1.626
268	6.342	6.305
269	1.713	3.493
270	1.247	14.351
271	1.432	4.352
272	1.148	4.175
273	1.371	6.160

TABLE 6

Absorption Rate Constants

<u>Subject</u>	<u>Formulation</u>		<u>Ratio</u>
	<u>A</u>	<u>B</u>	<u>A/B</u>
261	0.1690	0.1516	1.1148
262	0.3892	0.2137	1.8212
264	0.4952	0.5382	0.9201
265	0.2603	0.2579	1.0093
266	0.3291	0.1408	2.3374
267	0.2303	0.1240	1.8573
268	0.2228	0.1842	1.2096
269	0.1735	0.0958	1.8111
270	0.4068	0.3208	1.2681
271	0.2565	0.2118	1.2110
272	0.2678	0.3250	0.8240
273	0.2409	0.0725	<u>3.3228</u>
Mean			1.5589

TABLE 7

Ratio of Fractional Absorption

Subject	Formulation		Ratio A/B
	A	B	
261	0.4848	0.3341	1.4511
262	0.7024	0.5653	1.2425
264	0.9049	0.5647	1.6024
265	0.9126	0.6875	1.3274
266	0.5900	0.5666	1.0413
267	0.6624	0.6094	1.0852
268	0.4343	0.4058	1.0702
269	0.5871	0.5489	1.0692
270	0.5647	0.3540	1.5952
271	0.5684	0.5226	1.0874
272	0.6395	0.3105	2.0596
273	0.7764	0.6213	1.2500
Mean			1.3735

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APPENDIX I

Final Clinical Report
Experiment Number 10
Bio-Med Inc.
January 9, 1979

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CWZ 8-5-8 17

BIO - MED, Inc.

Tel: (202) 882 0377

January 9, 1979

FINAL REPORT

ANTIMALARIAL DRUG PROJECT


EXPERIMENT NUMBER 10

TITLE: COMPARATIVE BIOAVAILABILITY
AND PHARMACOKINETICS OF
WR 142,490·HCl (MEFLOQUINE
HYDROCHLORIDE) AND MEFLOQUINE
HYDROCHLORIDE·HLR

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FINAL REPORT

EXPERIMENT NUMBER 10

COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND
MEFLOQUINE HYDROCHLORIDE·HLR

TABLE OF CONTENTS

<u>SUBJECT</u>	<u>PAGE</u>
ABSTRACT	i
INTRODUCTION	1
METHODS AND MATERIALS	2
RESULTS	5
DISCUSSION	8
CONCLUSIONS AND RECOMMENDATIONS	10
EXPLANATION FOR POTENTIAL SUBJECTS	13
CONSENT FORM	17
TABLE I - Symptoms Summary	18
TABLE IIA - Laboratory Values, Normal Range	21
TABLE IIB - Abnormal Biochemical and Hematology Values	22
APPENDIX - Individual Subject Final Summary Sheets	25

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FINAL CLINICAL REPORT

EXPERIMENT NUMBER 10

COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE·HLR*

ABSTRACT

Mefloquine hydrochloride, a substituted quinoline methanol, has been shown to be an effective single dose agent in the treatment of chloroquine-resistant *P. falciparum* malaria and effective for prophylaxis. The drug administered to human subjects in single doses up to 1000 mg was well tolerated. Intolerance at higher doses was manifested by temporary light-headedness, diarrhea, abdominal cramps, occasional nausea and/or vomiting. Symptoms were considered dose related and mild in all cases.

A variety of formulations have been used in previous tolerance and therapeutic studies. Clinical results in infected volunteers and blood level determinations in a limited number of pharmacokinetic studies indicate considerable variation in the bioavailability of these formulations. The use of a new formulation in field studies, such as the HLR formulation, must therefore be supported by prior demonstration of adequate bioavailability.

A study for comparative bioavailability of the HLR formulation and an established effective formulation, WR 142,490·HCl, was done. A classical two way balanced crossover design including 3 groups of 4 subjects each was used. Symptoms were absent or mild and temporary. Symptomatology attributed to drug ingestion included gastrointestinal symptoms and headache following ingestion of both formulations. Lightheadedness occurred only following administration of the WR formulation. No significant changes in physical examination or laboratory values attributed to drug administration were observed. In conclusion, WR 142,490·HCl and the F. Hoffmann-La Roche, & Co. formulation were both well tolerated under conditions of this study. Drug assay analysis is not yet available and will be reported separately at a later date by the responsible institution.

*F. Hoffmann-La Roche, & Co.

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FINAL REPORT

EXPERIMENT NUMBER 10

COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND
MEFLOQUINE HYDROCHLORIDE·HLR*INTRODUCTION:

Mefloquine hydrochloride, a substituted quinoline methanol, has been shown to be an effective single dose agent in the treatment of chloroquine-resistant *P. falciparum* malaria. Its prophylactic effectiveness against chloroquine-resistant *P. falciparum* malaria inoculated by infected mosquitoes has also been demonstrated. Clinical studies in humans showed that 10 subjects who received 250 mg of WR 142,490·HCl weekly for 8 weeks were protected when exposed to mosquitoes heavily infected with multi-drug-resistant *P. falciparum*. The drug administered to human subjects in single doses up to 1000 mg was well tolerated. Intolerance at higher doses was manifested by temporary light-headedness, diarrhea, abdominal cramps, occasional nausea and/or vomiting. Symptoms were considered dose related and mild in all cases.

In addition, 12 healthy young males have completed a year long study during which each subject received a single weekly dose of 500 mg of WR 142,490·HCl without significant adverse clinical or laboratory effects. It appears that this anti-malarial is well tolerated and deserving of additional clinical investigations in man.

A variety of formulations have been used in previous tolerance and therapeutic studies. Clinical results in infected volunteers and blood level determinations in a limited number of pharmacokinetic studies indicate considerable variation in the bioavailability of these formulations. The use of a new formulation in field studies (such as the HLR formulation) must therefore be supported by prior demonstration of adequate bioavailability.

This study was designed to compare specific bioavailability parameters of WR 142,490·HCl and the HLR preparation of Mefloquine·HCl following single oral dose administration to healthy human male subjects, i.e.: peak blood levels, time to peak level, blood level-time patterns and area under concentration-time curve.

*HLR and WR as used in the text designate the F. Hoffmann-La Roche, & Co. and Walter Reed preparation respectively. Following a code number the letters designate the preparation administered.

The IND with Supplements and Clinical Summary were available at all processing committee meetings and were available at all times in the Office of the Clinical Director of BIO-MED, Inc.

METHODS AND MATERIALS:

Methods Subject Selection - Acceptability Criteria:

Thirteen healthy male subjects, 21 to 38 years of age, weighing 56-87 kg and within 10% of their ideal body weight, were employed for the study. They were recruited from the Washington, D.C. metropolitan area. Candidates were hired by BIO-MED, Inc. as temporary employees for study purposes.

Candidates for employment were screened to obtain the subjects for study. The medical evaluation included a comprehensive history and physical examination, chest X-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, and G6PD.

Subject acceptability criteria are based upon the precept that the risks of participation should be slight, and comparable for all subjects. Following this guideline, certain subjects were rejected routinely: for example, subjects with organic heart murmurs, splenomegaly or active lesions on chest X-ray. The presence of conditions which did not increase risk or potentially compromise the validity of the study, as illustrated by epidermophytosis, "shotty lymphadenopathy", or scarred tympanic membranes, was not ordinarily cause for rejection. Deviations of laboratory values of 3 standard deviations or more from the mean were generally cause for rejection dependent upon the particular test and associated clinical and laboratory observations. For example, a serum sodium of 153 mEq/L of itself would not be a cause for rejection, whereas a serum calcium of 11.2 mg/dl would.

Whenever doubt existed concerning the eligibility of a subject a decision was made following consultation with fellow M.D. investigators and other specialists, as appropriate. In this manner, questionable candidates were given full consideration and the integrity and ethics of the Research Team protected.

Qualified candidates were presented with a complete explanation of the background and procedures to be used in the study and all details of the protocol as it involved the individual subjects. They were interviewed in a group and individually in the presence of an investigator and a member of the Human Use Committee. Each participant was given the opportunity to ask questions. Following this, the consent form was read and those wishing to participate signed it in the presence of a witness, an investigator and a member of the Human Use Committee.

During the first 5 days of each study interval the subjects were housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center. Thereafter, they reported according to the protocol schedule (page 4).

Subject Assignment - Drug Administration:

A classical 2 way balanced crossover design was used. Each subject was randomly assigned to 1 of 2 possible sequences of drug administration, within the limitations of the design.

Three groups of 4 subjects each were admitted to the study sequentially. Within each group the 2 formulations were administered at a dose of 4 tablets* to 2 subjects each, by random assignment. Following a "wash out" period of 4 weeks, each subject was given 4 tablets* of the alternate formulation. Follow-up and sampling times following each administration are listed in the tables on page 11.

On the day of drug administration, subjects were permitted to drink water ad lib until 1 hour prior to and 2 hours after drug administration. Breakfast was withheld. The drug was administered in the presence of a member of the investigating team. Subjects returned to a regular diet 6 hours after drug administration.

The clinical and laboratory evaluation of the subjects is outlined on the following page:

*The 4 tablets for the Walter Reed formulation constituted a single dose of 1000 mg of Mefloquine·HCl, and for the F. Hoffmann-La Roche, & Co. preparation a single dose of 1100 mg Mefloquine·HCl. WR Lafayette Pharmacal lot number E-555; HLR lot number 21-5998-001-01.

SCHEMATIC STUDY PLAN FOR EACH DOSING

STUDY DAY	0*	1*	2*	3*	4*	5	7	8	14	21
Dose		X								
Physical Exam	X		X				X			X
Interview	X	X	X	X	X		X			X
Vital Signs	X	X	X	X	X		X			X
ECG	X		X				X			X
Laboratory Tests ⁺	X		X	X			X			X
Drug Assay ⁺⁺		X	X	X	X	X	X	X	X	X

*Controlled Environment in Study Unit 5-W.

⁺Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Carbon Dioxide, Uric Acid, Total Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alkaline Phosphatase, SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, Urinalysis. Additional studies were done as clinically indicated.

⁺⁺Drug Assay - Blood drawn on each subject immediately prior to drug administration and after dosing at 1,2,4,6,8,10,11, 12,13,15,20,24,28,34,48,56,63,72,76,80 hours and on days 5, 7,8,14, and 21.

The schedule for hematologic and biochemical blood tests is indicated. For these tests 27 ml venous blood was obtained while the subject was fasting before breakfast. Seven ml blood was used for determination of white blood cell and differential count, red blood cell count, hematocrit, hemoglobin, MCH, MCHC, MCV, and platelet count. Twenty ml of the venous blood specimen was centrifuged and the serum separated. The serum was divided into 2 samples. One sample was stored in the refrigerator as a "back-up" until the biochemical lab report was received. Thereafter it was stored in the freezer until released by the investigator. The other sample was used on the day obtained to determine the values for serum glucose, BUN, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, and total bilirubin.

On the last study day for each subject final physical and laboratory evaluation was done. All abnormal findings caused follow-up until normalcy, stabilization, or proper medical disposition was secured.

Drug Assay Specimen Collection and Time Schedule:

Specimen collection for drug assay was as follows: 6 ml venous blood was obtained in a heparin rinsed syringe. It was placed in 16 x 125 mm glass test tubes with screw-on teflon lined caps. Specimens were stored at -20°C pending transport to Department of Pharmacology, Walter Reed Army Institute of Research, for drug assay. A Specimen Collection Worksheet (BMI-WS-1) was completed by the staff nurse on each subject participating in the study. Twenty-four hour urine specimens were collected for drug assay on days 0, 1, and 2 from 8:01 a.m. to 8:00 a.m. (See the tables and Specimen Collection Worksheet on pages 11 and 12 respectively.)

RESULTS:

Subject code no. 263 withdrew from the study before administration of the WR preparation. Subject code no. 273 was substituted and the sequence of drug administration was inadvertently reversed.

Symptoms:

All symptoms are tabulated in Table I (pages 18 through 20). Three subjects (code nos. 265, 268, and 270) had symptoms possibly drug related following administration of each formulation. Four subjects (code nos. 261, 264, 266, and 271) had symptoms only after ingestion of the HLR preparation and 2 subjects (code nos. 262 and 272) only following administration of the WR formulation. Therefore, 7 of 13 administrations of the HLR formulation and 5 of 12 administrations of the WR preparation were associated with minor, possibly drug related symptoms.

Gastrointestinal:

The most frequent symptoms involved the gastrointestinal tract. Nausea variously described as a "queasy" feeling and "stomach ache" was associated with the administration of the HLR formulation on 2 occasions (subject code nos. 264 and 265) and the WR formulation on 3 occasions (subject code nos. 262, 265, and 268). Two other subjects receiving the HLR preparation had nausea not attributed to drug: 4 days after ingestion of the HLR preparation, subject code no. 262 had nausea

with 2 episodes of emesis. He was anorexic and dysphoric for approximately 1 week. Subsequently, a severe dental infection and abscess was found which was considered causal. Subject code no. 268 had transient lightheadedness and nausea associated with bloodletting 20 hours after administration of the HLR preparation not considered drug induced.

Loose stools occurred in seven subjects. Four subjects (code nos. 261, 265, 268, and 271) only following administration of the HLR preparation and 2 only following administration of the WR formulation. One subject had loose stools after receiving each formulation. Three subjects had onset of loose stools within 2 hours of drug administration: subject code nos. 261 and 268 after HLR and subject code no. 272 after the WR formulation. For subject (code no. 271) having 2 loose stools on the day of administration of the HLR preparation, the time of onset was not documented. One subject (code no. 266) had 1 loose stool 8 hours after receiving the WR formulation. Another subject (code no. 265) had 1 watery stool 48 hours after ingesting the HLR preparation. In 2 subjects with recurrent loose stools (code nos. 268 HLR and 270 WR) the frequency was 1 to 3 per day and the duration was 6 days except that subject code no. 268 HLR had abdominal cramps and passed 7 watery stools during the first 12 hours after onset at 45 minutes after dosing. In all other subjects the duration was of less than 24 hours or (code no. 265) only 1 loose stool was evacuated. The passage of watery stools was not associated with abdominal cramps except at onset in 1 subject (code no. 268 HLR).

In summary, 12 of 25 drug administrations were associated with mild, non-incapacitating gastrointestinal symptoms potentially attributable to drug administration: 7 episodes following HLR and 5 episodes following WR formulation administration.

Neurologic:

Eight of 25 drug administrations were associated with headache, lightheadedness, syncope, vasovagal reactions, or nightmares. However, only 2 subjects had symptoms considered potentially drug related: subject code no. 265 had headache and lightheadedness following administration of the HLR and WR formulation respectively. Additionally, subject code no. 268 had both symptoms only after receiving the WR preparation. Potentially drug related lightheadedness occurred as follows: subject code no. 265 WR noted onset of vague "dizziness" 4 hours after dosing with duration of 9 hours. The other subject with lightheadedness (code no. 268 WR) had been typing and reading constantly. Seven hours after dosing, he noted headache, lightheadedness, and nausea of 3 hours duration dissipating rapidly upon discontinuation of typing and reading.

Light-headedness not attributed to drug administration occurred on 3 occasions. Subject code no. 273 experienced light-headedness and syncope associated with bloodletting 30 minutes before dosing. Similarly, 2 subjects (code nos. 268 HLR and 272 WR) experienced transient vasovagal reactions associated with bloodletting 20 and 15 hours after dosing, respectively.

Three subjects in addition to subject code no. 268 WR noted headaches. One subject (code no. 265 HLR) experienced a possibly drug related headache. He noted onset of a mild right temporal headache 6 hours after dosing which persisted for 8 hours. Two other subjects had headaches not attributable to drug: 1 subject (code no. 261 WR) had a mild right temporal headache starting prior to dosing, of less than 24 hours duration. The other subject (code no. 263 HLR) had onset of a mild headache of approximately 15 minutes duration 6 days after dosing. These latter 2 subjects stated the headaches were similar to those they experienced commonly without obvious precipitants.

One subject (code no. 268 HLR) had vivid dreams and nightmares possibly but not probably drug related starting 56 hours after dosing and recurring nightly for 4 nights.

In summary, 1 subject (code no. 268) had dizziness, headache, and nausea of 3 hours duration with onset 7 hours after receiving the WR formulation, possibly precipitated or aggravated by typing and reading. Another subject (code no. 265) had vague "dizziness" for approximately 9 hours starting 4 hours after receiving the WR preparation. The same subject had a mild right temporal headache of approximately 8 hours duration starting 6 hours after receiving the HLR formulation. In both subjects the neurologic symptoms potentially drug related were mild and temporary. Neurologic symptoms otherwise were not considered potentially drug related.

Miscellaneous:

Three subjects (code nos. 264, 272, and 273) had symptoms of the common cold during the interval of study. Three other subjects (code nos. 262, 267, and 268) had symptoms and signs not considered related to drug administration which are presented in Table I and the individual subject final summaries.

Physical Findings:

No changes attributed to drug administration occurred in any subject.

Laboratory Values:

The range ($\bar{x} \pm 2$ SD) of laboratory values for the BIO-MED, Inc. normal population ($n > 100$) is presented as Table IIA (page 21). All laboratory values outside the normal range are included in Table IIB (pages 22 through 24). All subjects had 2 or more values outside the normal range reported. Minimal serum carbon dioxide content elevation reported in 9 subjects was the most frequently reported abnormality. The elevations were minimal and inconsistent, occurring before and after administration of both formulations without discernable pattern. This observation of minimal, inconsistent deviations of laboratory values in a random manner is characteristic of most of the abnormalities reported and not considered drug related. In some subjects values for a given determination clustered about the upper or lower limits of the range for that determination. Subject code no. 264 exemplifies this occurrence for serum creatinine and albumin values. Subject code no. 273 demonstrated a persistent minimal elevation of serum alkaline phosphatase before and after administration of both formulations. In the absence of other laboratory or clinical findings to suggest an active disease process, the observation of a fixed pattern of deviation before and after drug administration is not considered drug related.

Two subjects (code nos. 261 and 266) demonstrated minimal SGPT elevations following administration of the HLR formulation: an isolated elevation to 56 U/L occurred 6 days after dosing in subject code no. 261 and elevations to 52 U/L and 48 U/L occurred 2 and 20 days after dosing respectively in subject code no. 266 with a normal value of 38 U/L reported 6 days after dosing. The upper limits for this determination is 47 U/L. These minimal and inconsistent deviations may be considered possibly, but not probably drug related.

Electrocardiograms:

No significant changes in serial rhythm tracings occurred in any subject.

DISCUSSION:

The 2 formulations of mefloquine were well tolerated at the dose levels administered. Five of 12 administrations of the WR formulation and 7 of 13 administrations of the HLR preparation were associated with symptoms. Three subjects had symptoms following administration of each drug, four only associated with the HLR formulation, and 2 only associated

with the WR preparation. The character and frequency of symptoms suggest no significant difference in clinical response to the 2 formulations. Therefore, symptoms potentially drug related occurred in 13 of the 25 trials. The symptoms were mild and did not interfere with normal activity.

The absence of gastrointestinal symptoms in previous WR 142,490·HCl safety and tolerance studies at comparable dose levels suggests their frequency in the current study may be attributed in part to the requirements of the experimental protocol. The subjects in this study ingested the drug following an overnight fast and continued to fast for 6 hours following drug administration. During this interval frequent bloodletting was required for specimen collection. These factors may have been causal or contributory to the gastrointestinal symptomatology described.

Light-headedness, headache, and nightmares were observed in association with WR 142,490·HCl administration in previous studies at dose levels higher than those administered in this study. Their frequency in this study was 3 potentially drug related involving 2 subjects in 25 trials: 2 after administration of the WR formulation and 1 after administration of the HLR preparation. The symptoms were mild, temporary, and not incapacitating. The conditions of the study rather than drug effect may have been causal or contributed to the neurologic symptoms.

Intercurrent illnesses and medical conditions unrelated to drug administration occurred with the frequency expected in a study of this duration.

Similarly deviations of laboratory values not attributable to drug administration were frequent. Two subjects receiving the HLR preparation had minimal elevation of SGPT values reported considered possibly, but not probably drug related.

In summary, the study has been completed without significant deviations or adverse reactions and the specimens for assay are in custody of the responsible organization.

CONCLUSIONS AND RECOMMENDATIONS:

This study was performed for pharmacokinetic purposes. Noted as an integral part of monitoring was the occurrence of mild and temporary gastrointestinal and neurologic symptoms considered related primarily to prolonged fasting and frequent bloodletting.

The drug assay results will be reported at a later date by the responsible organization.

EXPERIMENT 10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF
of WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) and
MEFLOQUINE HYDROCHLORIDE·HLR

DRUG ASSAY COLLECTION

DAY OF THE STUDY	SPECIMEN NUMBER	BLOOD SPECIMEN TIME	BLOOD VOLUME (ml)
1	1	0 Prior to dosing	6
	2	1 hr 9:00 AM	6
	3	2 hr 10:00 AM	6
	4	4 hr NOON	6
	5	6 hr 2:00 PM	6
	6	8 hr 4:00 PM	6
	7	10 hr 6:00 PM	6
	8	11 hr 7:00 PM	6
	9	12 hr 8:00 PM	6
	10	13 hr 9:00 PM	6
	11	15 hr 11:00 PM	6
2	12	20 hr 4:00 AM	6
	13	24 hr 8:00 AM	6
	14	28 hr NOON	6
	15	34 hr 6:00 PM	6
3	16	48 hr 8:00 AM	6
	17	56 hr 4:00 PM	6
	18	63 hr 11:00 PM	6
4	19	72 hr 8:00 AM	6
	20	76 hr NOON	6
	21	80 hr 4:00 PM	6
HOME			
5	22	96-98 hr 8:00-10:00 AM	6
7	23	144-146 hr 8:00-10:00 AM	6
8	24	168-170 hr 8:00-10:00 AM	6
14	25	312-314 hr 8:00-10:00 AM	6
21	26	480-482 hr 8:00-10:00 AM	6
TOTAL			156 (ml)

URINE SPECIMENS		
0	1	8:01 AM to 8:00 AM
1	2	8:01 AM to 8:00 AM
2	3	8:01 AM to 8:00 AM

TOTAL AMOUNT OF BLOOD WITHDRAWN FOR EACH STUDY SUBJECT
FOLLOWING EACH DRUG ADMINISTRATION

	HEMATOLOGY	CHEMISTRY	BLOOD ASSAY	TOTAL (ml)
DAY 0	7	20		27
1			66	66
2	7	20	24	51
3	7	20	18	45
4			18	18
5			6	6
7	7	20	6	33
8			6	6
14			6	6
21			6	6
TOTAL				264 (ml)

Specimen Collection Worksheet

Experiment #10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND
MEFLOQUINE HYDROCHLORIDE·HLR

Name: _____ Code: _____ Age: _____ Ht: _____ cm. Wt: _____ k

First Dosing

Drug Formulation: _____
Total Dose: _____ mg Dose(mg/kg): _____
Date & Time Dosed: _____

Second Dosing

Drug Formulation: _____
Total Dose: _____ mg Dose(mg/kg): _____
Date & Time Dosed: _____

Whole Blood Collection

Spec. No.	P.D.*	Sched	Actual
1	-15 min.		
2	1 hr		
3	2 hr		
4	4 hr		
5	6 hr		
6	8 hr		
7	10 hr		
8	11 hr		
9	12 hr		
10	13 hr		
11	15 hr		
12	20 hr		
13	24 hr		
14	28 hr		
15	34 hr		
16	48 hr		
17	56 hr		
18	63 hr		
19	72 hr		
20	76 hr		
21	80 hr		
22	96-98 hr		
23	144-146 hr		
24	168-170 hr		
25	312-314 hr		
26	430-482 hr		

Whole Blood Collection

Spec. No.	P.D.*	Sched	Actual
1	-15 min.		
2	1 hr		
3	2 hr		
4	4 hr		
5	6 hr		
6	8 hr		
7	10 hr		
8	11 hr		
9	12 hr		
10	13 hr		
11	15 hr		
12	20 hr		
13	24 hr		
14	28 hr		
15	34 hr		
16	48 hr		
17	56 hr		
18	63 hr		
19	72 hr		
20	76 hr		
21	80 hr		
22	96-98 hr		
23	144-146 hr		
24	168-170 hr		
25	312-314 hr		
26	480-482 hr		

24 Hr. Urine Collections

STUDY DAY	TIME		TOTAL		Vol (ml)
	Begin	End	Hrs.	Min.	
0	+	0800			
1	0801	0800			
2	0301	0800			

24 Hr. Urine Collections

STUDY DAY	TIME		TOTAL		Vol (ml)
	Begin	End	Hrs.	Min.	
0	+	0800			
1	0801	0800			
2	0801	0800			

* Post Dose

BMI-WS-1

+Variable fraction of 24 hour
collection due to starting times.

BIO - MED, Inc.

Tel: (202) 882-0977

EXPLANATION FOR POTENTIAL SUBJECTS

ANTIMALARIAL DRUG PROJECT

EXPERIMENT NUMBER 10

Comparative Bioavailability and Pharmacokinetics
of WR 142,490·HCl (Mefloquine Hydrochloride)
and Mefloquine Hydrochloride·HLR

GENTLEMEN:

This document explains the nature of the study, its purpose, procedures, risks and benefits. You will be given the opportunity after reading it to ask additional questions. If you then choose to participate as a research subject, you will be asked to initial the last page signifying that you have read and understand its contents prior to obtaining your formal written consent to participate in the study. The subject will participate for a total of 50 days.

This study involves taking by mouth the drug mefloquine hydrochloride. Mefloquine is similar to quinine and has been approved by the Food and Drug Administration for investigational studies. The drug has been administered to more than 100 subjects to determine its safety and tolerance, and ability to prevent and cure malaria.

Previous studies with this drug established that it was well tolerated in single doses up to 1000 mg. Doses of 1250, 1500, and 1750 mg occasionally caused transient light-headedness or gastrointestinal symptoms. At 1250 mg only mild diarrhea lasting for $\frac{1}{2}$ to 3 hours after taking the drug was reported. There was no associated nausea, vomiting or abdominal pain. At 1500 mg two subjects reported a transient sense of light-headedness, two subjects had mild diarrhea without other symptoms, and one subject vomited five minutes after taking the drug. At 1750 mg there was mild to moderate diarrhea, with the number of stools varying from 1 to 6 over a period of 35 minutes to 6 hours after taking the drug. Occasional nausea and mild abdominal cramps were also reported by some subjects.

This study is to compare the bioavailability of two different formulations. It will be conducted using one tablet formulation provided by Walter Reed Army Institute of Research, and the other provided by F. Hoffman-La Roche, & Co. The dose to be administered in both cases is 4 tablets*. You may experience some of the symptoms discussed above. No other symptoms or long term effects are anticipated.

*The four tablets for the Walter Reed formulation will constitute a single dose of 1000 mg of Mefloquine·HCl, and for the F. Hoffman-La Roche, & Co. preparation a single oral dose of 1100 mg Mefloquine·HCl.

Research Facility: George Hyman Research Building • 110 Irving Street, N. W. • Washington, D.C. 20010
Mailing Address: 1295 Lavall Drive • Gambrills, Maryland 21054

We will study these two preparations by using a two way cross-over design. You will be assigned in a random manner to one of 3 groups designated Group I through III, each group containing 4 subjects. You will receive one drug preparation on one occasion and four weeks later the other formulation. You will be required to remain on the research unit for five days at the beginning of each period.

On the day the drug is to be administered, breakfast will be withheld. You will be permitted to drink water as you wish except for 3 hours encompassing the time of drug administration. The drug will be administered in the presence of a member of the investigating team at 8:00 AM. At 2:00 PM you may resume the normal diet you select from the hospital menu until you are discharged from the unit.

The purpose of the present study is to accurately determine the pattern in which the drug appears and disappears from your body. Blood (6 ml) will be drawn at the times specified in Table II after you take the drug to measure the amount of drug present. You will notice that the frequency of specimens is much greater during the early days of the study. In addition, we will collect all of your urine during the first 3 days encompassing the time of drug administration. You will be admitted to the research unit for the first 5 days and seen on brief visits for a period of 3 weeks. On the fourth week you will be readmitted to the research unit for a second 5 day period, after which you will be seen on brief visits thereafter per schedule. The long period of follow-up is required because of the expected slow release of the drug from the body. It is important that the blood be obtained as nearly as possible to the times specified in Table II and that you eat a light breakfast (i.e. cereal, milk, juice, coffee, bread -- no eggs or bacon) on the days you come in for blood drawing. It is also important that you avoid taking any other medication during the entire period and avoid the use of alcohol. Such factors as time of day, meals, alcohol, other drugs, and lack of proper sleep may affect the level of drug in your blood on any given day.

It is expected that the amount of drug remaining in your blood on the last day of the study will be very low. However, the late specimens are just as important as the early specimens in obtaining an overall accurate assessment of the way the drug is handled by the body. Therefore, please do not start the study if you anticipate difficulty in adhering to the schedule.

C
The amount of blood to be withdrawn for the entire study will be 555 ml, which is obtained over a seven week period and is about 39 ml more than a unit of whole blood that many people donate at American Red Cross Center Blood Banks. Instead of repeated venipunctures we will place a small teflon catheter in an arm vein and obtain the blood samples from it during the first fifteen (15) hours. In this way repeated venipunctures may not be necessary. The specifics for the study are presented in the schematic on the following page.

SCHEMATIC STUDY PLAN FOR EACH DOSING

DAY OF STUDY	0*	1*	2*	3*	4*	5	7	8	14	21
Dose		X								
Physical Exam	X		X				X			X
Interview	X	X	X	X	X		X			X
Vital Signs	X	X	X	X	X		X			X
ECG	X		X				X			X
Laboratory Tests ⁺	X		X	X			X			X
Drug Assay ⁺⁺		X	X	X	X	X	X	X	X	X

*Controlled Environment

⁺Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Carbon Dioxide, Uric Acid, Total Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alkaline Phosphatase, SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, and Urinalysis. Additional studies will be done as clinically indicated.

⁺⁺Drug Assay - Each subject immediately prior to drug administration and after dosing at 1, 2, 4, 6, 8, 10, 11, 12, 13, 15, 20, 24, 28, 34, 48, 56, 63, 72, 76, 80 hours and on days 5, 7, 8, 14, and 21.

On the days indicated by *, the participants in the study will remain in a controlled environment. The entire group will remain together with a member of the Research Unit Staff and will function according to their direction. Facilities provided while participating in the study include room and board with a study-lounge area. Recreation (tennis and basketball) is also provided if weather conditions permit and supervision is available.

You have already had many of the examinations listed on the schematic as a part of your qualification examination. The laboratory tests require urine collections and venipunctures to obtain blood specimens. This will not affect you except for temporary discomfort associated with obtaining blood from your arm vein.

On the last study day for each subject final physical and laboratory evaluation will be done. All abnormal findings will cause follow-up until normalcy, stabilization or proper medical disposition is secured.

The Human Use Committee members are also looking after your safety. They insure that you are not subjected to undue risk and discomfort. A member of this committee will be available to speak with you at the Washington Hospital Center. After members of the investigating team and Human Use Committee are satisfied that you understand the study and written informed consent form you will be permitted to sign it. No subject may participate without a signed consent. By signing the informed consent you signify that the study has been explained to you with regard to its risks and requirements and you wish to participate.

It should be clear that your participation in this study is of no therapeutic value to you personally. The benefit, rather, is to others who live in parts of the world where malaria is a serious problem, and to Americans, civilian and military, who may travel to these areas. For this reason especially, your participation must be entirely voluntary with full knowledge of the personal risks and general benefits involved. Furthermore, you retain the right to withdraw your consent at any time without prejudice.

If after reading this document, you have any additional questions, please ask them before affixing your initials below and signing the consent form.

Initials of Participant

SUBJECT AGREEMENT

CONSENT TO PARTICIPATE AS A STUDY SUBJECT

I, _____, hereby give my informed consent to participate as a study subject in the study entitled, "Comparative Bioavailability and Pharmacokinetics of WR 142,490·HCl (Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR."

The implications of my voluntary participation; the nature, duration and purpose; the methods by which it is to be conducted and the inconveniences and hazards which may reasonably be expected have been explained to me by Dr. _____, and are set forth in the document entitled, "EXPLANATION: ANTIMALARIAL DRUG PROJECT EXPERIMENT NUMBER 10, Comparative Bioavailability and Pharmacokinetics of WR 142,490·HCl (Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR," which I have signed.

I understand that with all drug administration and clinical investigation there are associated potential discomforts and risks. The discomforts and potential risks of participation as a subject in this study have been explained to me and I freely and voluntarily accept them. I understand that I will attain no direct therapeutic benefits from participation in the study.

All questions and inquiries I have made regarding the study have been answered to my satisfaction and I understand that I have the right to ask questions concerning the study at any time and have them answered to my satisfaction. Further, I understand I am free to withdraw, without prejudice, my consent and participation from the project at any time; however, I may be requested to undergo further examination, if in the opinion of the attending physician, such examinations are necessary for my health or well-being.

I consent to the taking and publications of any photographs in the course of the study for the purpose of advancing medical science, provided that my identity will remain confidential.

I certify that I have read and understand the above consent and that the explanations therein were made to me and that all inapplicable paragraphs, if any, were stricken before I signed.

Date

Witness - Human Use Comm. Cert.

Signature

Investigator Certification

Address

Witness

REAFFIRMATION OF CONSENT:

Date

Witness

Signature

EXPERIMENT NO. 10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF
WR 142,490-HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE-HLR

TABLE I: SYMPTOMS SUMMARY

GROUP	CODE NUMBER	FORMULATION		Nausea		Loose Stool		Headache		Light-headedness		Other†	
		1st Dose	2nd Dose	A*	B*	A	B	A	B	A	B	A	B
I	261		HLR†										
		WR†											
	262	HLR		96.0	72.0			Right temporal -1.0	< 24.0			Emesis x 2 108.0	122.0
			WR	1.0	5.0							Anorexic, dysphoria 96.0	168.0
263	HLR							Chronic 144.0	≈ 0.15			Pale, hyperventilation, after shower 54.0	0.3
								NOT DOSED				Pain, dental abscess 336.0	192.0
264	HLR			"Queasy" feeling 0.45	1.15								
	WR											Mild coryza 504.0	≈ 96.0

*A = Onset hours . minutes after dosing: (-) before dosing

*B = Duration hours . minutes after onset

†See Individual Subject Final Summary for details:

-HLR is designated as Formulation A

-WR is designated as Formulation B

TABLE I: SYMPTOMS SUMMARY (cont.)

GROUP	CODE NUMBER	FORMULATION		Nausea		Loose Stool		Headache		Light-headedness		Other [†]	
		1st Dose	2nd Dose	A*	B*	A	B	A	B	A	B	A	B
II	265		HLR [†]	"Queasy stomach" -1.0	≈ 8.0	Watery stool x 1 48.0	< 0.05	Right temporal 6.0	≈ 8.0				
		WR [†]		Stomach ache 3.30	≈ 0.20					"Dizziness" 4.0	≈ 9.0		
	266		HLR					NO SYMPTOMS					
		WR				Watery stool x 1 ≈ 8.0	< 0.05						
	267	HLR						NO SYMPTOMS					
III	268		HLR	2° to bloodletting 20.0	0.05	7 Day 1, then 1-2/day 0.45	144.0			2° to bloodletting 20.0	0.05	Perianal pain - fissures ≈ 168.0	≈ 168.0
		WR		7.0	3.0 [†]			7.0	3.0 [†]	"Dizziness" 7.0	3.0 [†]	Vivid dreams, nightmares 56.0	96.0
	269	HLR						NO SYMPTOMS				Pain in inguinal ring 60.0	≈ 2.0
		WR						NO SYMPTOMS					

*A = Onset hours . minutes after dosing: (-) before dosing
 *B = Duration hours . minutes after onset

[†]See Individual Subject Final Summary for details:
 -HLR is designated as Formulation A
 -WR is designated as Formulation B

TABLE I: SYMPTOMS SUMMARY (cont.)

GROUP	CODE NUMBER	FORMULATION		Nausea		Loose Stool		Headache		Light-headedness		Other [†]	
		1st Dose	2nd Dose	A*	B*	A	B	A	B	A	B	A	B
III	270	HLR [†]											
			WR [†]			Watery stool x 1 6.0	< 0.05						
	271		HLR			Recurrent 2-3x / day 6.0	144.0						
		WR				Watery stools x 2 unrecorded	< 24.0	NO SYMPTOMS					
I	272		HLR									Mild coryza & sore throat -24.0	144.0
		WR				Recurrent x 2 1.0	2.0					Vaso-vagal reaction at bloodletting 15.0	0.6
	273		HLR					NO SYMPTOMS					
		WR										Mild coryza "common cold" 144.0	< 168.0
												Vaso-vagal reaction at bloodletting -0.50	< 0.30

*A = Onset hours . minutes after dosing: (-) before dosing

*B = Duration hours . minutes after onset

[†]See Individual Subject Final Summary for details:

-HLR is designated as Formulation A

-WR is designated as Formulation B

TABLE IIA: LABORATORY VALUES, NORMAL RANGE

April - June 1975

AMDP Subjects

Age: 21-45 Sex: Male Normal Population: n > 100

Laboratory: National Health Laboratories

	\bar{x}	S.D.	± 2 S.D.
Glucose (mg/dl)	90	12	66-114
BUN (mg/dl)	15	3.1	9-21
Creatinine (mg/dl)	1.1	0.16	0.8-1.4
Sodium (mEq/L)	144	3.5	137-151
Potassium (mEq/L)	4.4	0.41	3.6-5.2
Chloride (mEq/L)	104	3.0	98-110
Carbon Dioxide (mEq/L)	27	1.9	23-31
Uric Acid (mg/dl)	6.0	1.0	4-8
Total Protein (g/dl)	7.2	0.41	6.4-8.0
Albumin (g/dl)	4.6	0.25	4.1-5.1
Globulin (g/dl)	2.6	0.42	1.8-3.4
Calcium (mg/dl)	10	0.44	9.0-10.9
Phosphate (mg/dl)	3.5	0.50	2.5-4.5
Cholesterol (mg/dl)	176	33	110-242
Triglyceride (mg/dl)	111	58	0-207
Alkaline Phosphatase (U/L)	60	17	26-94
SGOT (U/L)	23	12	0-47
SGPT (U/L)	17	15	0-47
LDH (U/L)	153	40	73-233
Total Bilirubin (mg/dl)	0.58	0.34	0.0-1.3
Hematocrit (Vol %)	45	2.52	40-50
Hemoglobin (GMS %)	15	0.84	13.3-16.7
WBC (thous/cu mm)	6.3	1.6	3.1-9.5
RBC (million/cu mm)	5.0	0.36	4.3-5.7
Lymph (%)	39	9.8	19-59
Seg. Neutrophils (%)	58	11	36-80
MCV (cu microns)	90	3.85	82-98
MCHC (%)	33	2.0	29-37
MCH (micro micro GM)	30	1.45	27-33
Platelet Count (thous/cu mm)	264	81	102-426

EXPERIMENT NO. 10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF
WR 142,490-HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE-HLR

TABLE IIB: ABNORMAL BIOCHEMICAL AND HEMATOLOGY VALUES

GROUP- CODE NUMBER	FORMULATION		TESTS	FIRST DOSE STUDY DAY						SECOND DOSE STUDY DAY						NORMAL RANGE			
	1st Dose	2nd Dose		Screen															
					0	2	3	7	0	2	3	7	21						
I- 261	WR ⁺	HLR ⁺	Glucose	97	105	107	119*	97	96	103	124*	108	92	66-114 mg/dl					
			Triglyceride	88	117	153	167	188	210*	128	182	284*	220*		0 - 207 mg/dl				
			SGPT	40	37	35	30	42	32	33	35	56*	39			0 - 47 U/L			
			Eosinophiles	5	2	4	5	2	3	7*	3	3	4				0 - 5 %		
I- 262	HLR	WR	Carbon Dioxide	35*	33*	34*	33*	31	32*	33*	34*	29	28	23 - 31 mEq/l					
			Total Protein	7.2	6.8	6.3*	6.3*	7.1	7.2	6.5	7.5	7.1	7.1		6.4 - 8.0 g/l				
			Albumin	4.6	4.3	3.9*	4.0*	4.5	4.2	4.0*	4.6	4.4	4.4			4.1 - 5.1 g/l			
			White Blood Cell Count	6.7	5.4	7.3	6.8	11.0*	6.2	5.8	5.9	6.8	11.2*				3.1-9.5 th/c		
I- 263	HLR	-	Carbon Dioxide	28	30	29	28	32*	-	-	-	-	23 - 31 mEq/l						
			Uric Acid	4.6	5.2	5.5	4.5	3.6*	-	-	-	-		-	4 - 8 mg/dl				
			Albumin	4.3	4.0*	4.2	4.2	4.2	-	-	-	-		-		4.1 - 5.1 g/l			
I- 264	WR	HLR	Creatinine	0.7*	0.7*	0.5*	0.5*	0.6*	0.6*	0.6*	0.5*	0.7*	0.5*	0.8 - 1.4 mg/l					
			Albumin	4.2	4.2	3.8*	4.1	4.5	4.4	4.0*	4.3	4.4	4.2		4.1 - 5.1 g/l				
II- 265	WR	HLR	Carbon Dioxide	33*	29	35*	33*	33*	30	34*	32*	32*	34*	34*		23 - 31 mEq/l			
			Total Protein	6.5	6.5	6.5	6.0*	6.4	6.6	6.8	6.4	6.6	6.5	6.4 - 8.0 g/l					
			Albumin	4.2	4.3	4.2	4.1	4.0*	4.3	4.5	4.1	4.2	4.1		4.1 - 5.1 g/l				
			Cholesterol	232	242	258*	210	207	243*	249*	234	226	236				110 - 242 mg/l		
			Alkaline Phosphatase	113*	100*	97*	93	99*	101*	107*	97*	104*	95*					26 - 94 U/L	
			LDH	205	192	194	146	190	194	168	173	182	235*						73 - 233 U/l
			Eosinophiles	1	0	2	1	3	1	6*	2	0	4						
II- 266	WR	HLR	Carbon Dioxide	31	27	31	31	30	28	27	27	29	32*			23 - 31 mEq/l			
			SGPT	20	24	36	36	20	25	37	52*	38	48*	0 - 47 U/L					

*denotes abnormality

†HLR is designated as Formulation A and WR as Formulation B in Individual Subject Final Summaries

TABLE IIB: ABNORMAL BIOCHEMICAL and HEMATOLOGY VALUES (cont.)

GROUP- CODE NUMBER	FORMULATION		TESTS	FIRST DOSE STUDY DAY					SECOND DOSE STUDY DAY					NORMAL RANGE
	1st Dose	2nd Dose		Screen	0	2	3	7	0	2	3	7	21	
II- 267	HLR [†]	WR [†]	Carbon Dioxide	33*	26	32*	28	31	28	28	32*	29	34*	23 - 31 mEq
			Hemoglobin	16.0	16.0	16.4	16.9*	14.9	16.0	16.8*	17.1*	15.5	16.4	13.3-16.7 g
			White Blood Cell Count	7.2	7.6	8.3	9.0	6.9	10.4*	8.3	7.6	6.6	6.7	3.1-9.5 th/ μ
			Lymphocytes	24	20	23	20	17*	23	18*	16*	23	22	19 - 59 %
			Eosinophiles	6*	1	8*	2	3	2	4	6*	2	6*	0 - 5 %
II- 268	HLR	WR	Carbon Dioxide	34*	27	31	32*	31	28	28	30	28	34*	23 - 31 mEq
			Phosphate	2.7	2.3*	2.3*	2.6	3.4	2.7	2.6	2.8	2.8	4.1	2.5 - 4.5 m
			Hematocrit	47.8	45.4	48.8	50.7*	43.7	43.9	48.0	47.4	45.3	45.3	40 - 50 Vol
			Eosinophiles	0	0	2	2	1	4	6*	0	0	2	0 - 5 %
			% ATL	0	0	0	0	0	0	0	0	0	8*	0 %
III- 269	HLR	WR	Glucose	74	84	85	80	72	50*	82	84	83	92	66 - 114 mg
			Carbon Dioxide	34*	32*	31	32*	30	30	28	29	30	25	23 - 31 mEq
			Triglyceride	132	161	89	139	110	219*	142	139	103	218*	0 - 207 mg/100
III- 270	HLR	WR	Carbon Dioxide	34*	34*	30	32*	31	30	27	29	32*	33*	23 - 31 mEq
			Phosphate	2.5	2.3*	2.8	2.7	2.6	2.5	2.7	2.7	2.9	2.6	2.5 - 4.5 m
			Eosinophiles	6*	3	2	2	7*	7*	3	5	4	4	0 - 5 %
III- 271	WR	HLR	Carbon Dioxide	30	29	30	32*	27	28	27	30	29	30	23 - 31 mEq
			Albumin	4.5	4.7	4.3	4.4	4.2	4.0*	4.2	4.7	4.3	4.4	4.1 - 5.1 g
			LDH	223	220	164	166	174	202	168	182	188	246*	72 - 233 U/l
			Total Bilirubin	0.7	0.5	1.0	0.8	1.4*	0.3	0.5	1.1	0.6	0.7	0.0 - 1.3 m
			Lymphocytes	28	30	28	28	30	28	32	14*	28	26	19 - 59 %
			Segmenters	68	64	60	70	68	68	58	84*	68	73	36 - 80 %
MCH	32.8	31.1	32.5	32.6	31.5	31.6	32.8	33.1*	32.3	32.6	27-33 micro			

*denotes abnormality

†HLR is designated as Formulation A and WR as Formulation B in Individual Subject Final Summaries

TABLE IIB: ABNORMAL BIOCHEMICAL and HEMATOLOGY VALUES (cont.)

GROUP- CODE NUMBER	FORMULATION		TESTS	FIRST DOSE STUDY DAY							SECOND DOSE STUDY DAY							NORMAL RANGE
	1st Dose	2nd Dose		Screen	0	2	3	7	0	2	3	7	21					
III- 272	WR ⁺	HLR ⁺	Glucose	75	93	98	91	87	87	93	91	125*	85	66 - 114 mg/dl 6.4 - 8.0 g/dl 4.1 - 5.1 g/dl 19 - 59 % 0 - 5 % 0 % 0 - 6 %				
			Total Protein	6.2*	6.5	6.4	6.7	6.6	6.2*	6.3*	6.5	6.3*	6.5					
			Albumin	4.2	4.1	4.1	4.4	4.1	3.9*	4.1	4.4	4.1	4.2					
			Lymphocytes	30	40	18*	34	40	28	24	33	34	48					
			Eosinophiles	4	2	6*	4	2	4	5	0	4	4					
			% ATL	0	0	0	0	0	0	3*	0	0	0		0			
I- 273	WR	HLR	Monocytes	2	4	8*	0	5	3	0	2	3	0					
			Glucose	98	86	92	93	125*	117*	96	108	100	107					
			Carbon Dioxide	31	24	30	30	27	26	32*	27	31	28					
			Albumin	4.1	4.4	4.1	4.2	4.1	3.8*	4.1	4.4	4.1	4.0*					
			Calcium	10.4	10.1	9.9	10.2	9.9	8.7*	9.7	10.2	9.3	9.3					
			Alkaline Phosphatase	103*	115*	101*	96*	107*	99*	101*	104*	96*	101*					
26 - 94 U/L																		

denotes abnormality

HLR is designated as Formulation A and WR as Formulation B in Individual Subject Final Summaries

APPENDIX II

Sample Times and Blood Concentrations
of Merfloquine

PHCC 84 0083
DW 15-8 18

Experiment #10

Deepak Bakhu #251 Age 21 Ht 5' 10" Wt 150 lbs

First Dosing formulation B Lafayette
Dosed on 11/18/78 0815

Serial No.	Time Post Dose	Actual Time	Count per gram
1	0.5 min	0805	50
2	1 min	0815	50
3	1.5 min	0825	57
4	2 min	0835	61
5	2.5 min	0845	100
6	3 min	0857	107
7	3.5 min	0910	103
8	4 min	0912	104
9	4.5 min	0925	105
10	5 min	0935	140
11	5.5 min	0940	100
12	6 min	0950	100
13	6.5 min	0952	107
14	7 min	1003	105
15	7.5 min	1007	115
16	8 min	1012	108
17	8.5 min	1017	107
18	9 min	1022	109
19	9.5 min	1025	101
20	10 min	1028	101
21	10.5 min	1045	101
22	11 min	1046	101
23	11.5 min	1052	101
24	12 min	1047	101

Experiment 11

Deepak Bakshi #261 - continued

Second-dosage formulation of Hoffman-Laurance
Dosed on 7/28/98 11:00

Dose no.	Time Post Dose	Actual Time	Area Under Curve
1	15 min	0740	0
	0 time	0800	0
	1 hr	0900	132
	2 hr	1005	147
	4 hr	1100	213
	6 hr	1201	271
	8 hr	1305	320
	10 hr	1315	371
	11 hr	1303	384
	12 hr	1401	401
	13 hr	1501	411
	15 hr	1558	441
	17 hr	1654	474
	19 hr	1756	504
	20 hr	1856	525
	24 hr	1907	557
2	25 hr	2001	471
	26 hr	2101	481
	27 hr	2201	491
	28 hr	2301	501
	29 hr	2401	511
	30 hr	2501	521
	31 hr	2601	531
	32 hr	2701	541
	33 hr	2801	551
	34 hr	2901	561
3	40 hr	3001	571
	41 hr	3101	581
	42 hr	3201	591
	43 hr	3301	601

0-279-7687-1 \$12

None Skop #262 Age 1 Wt 13.4 Lb Wt 15.5 Lb

First dosing formulation = 100 mg/ml - 300 mg/ml

closed on 1-21-78 6000

LINE NO.	TIME	DATE	TIME	TIME	TIME
15	110	110	110	110	110
16	110	110	110	110	110
17	110	110	110	110	110
18	110	110	110	110	110
19	110	110	110	110	110
20	110	110	110	110	110
21	110	110	110	110	110
22	110	110	110	110	110
23	110	110	110	110	110
24	110	110	110	110	110
25	110	110	110	110	110
26	110	110	110	110	110
27	110	110	110	110	110
28	110	110	110	110	110
29	110	110	110	110	110
30	110	110	110	110	110
31	110	110	110	110	110
32	110	110	110	110	110
33	110	110	110	110	110
34	110	110	110	110	110
35	110	110	110	110	110
36	110	110	110	110	110
37	110	110	110	110	110
38	110	110	110	110	110
39	110	110	110	110	110
40	110	110	110	110	110
41	110	110	110	110	110
42	110	110	110	110	110
43	110	110	110	110	110
44	110	110	110	110	110
45	110	110	110	110	110
46	110	110	110	110	110
47	110	110	110	110	110
48	110	110	110	110	110
49	110	110	110	110	110
50	110	110	110	110	110

Experiment #10

Drug: 5000 (2000 cont. used)

Second dosing formulation: 1. Larasette
Dosed on 11/18/13 10:10

Time	Post-Dose	Actual Time	Conc. (ng/ml)
-15 min		0745	49
0 time		0810	
1 hr		0908	101
2 hr		1010	423
4 hr		1104	406
6 hr		1145	373
8 hr		1200	382
10 hr		1207	300
11 hr		1211	181
12 hr		1214	170
13 hr		1217	0.390
14 hr		1217	393
15 hr		1217	339
16 hr		1217	355
17 hr		1217	146
18 hr		1217	161
19 hr		1217	130
20 hr		1217	300
21 hr		1217	110
22 hr		1217	397
23 hr		1217	350
24 hr		1217	111
25 hr		1217	0.390
26 hr		1217	110
27 hr		1217	390
28 hr		1217	113
29 hr		1217	390
30 hr		1217	113
31 hr		1217	390
32 hr		1217	113
33 hr		1217	390
34 hr		1217	113
35 hr		1217	390
36 hr		1217	113
37 hr		1217	390
38 hr		1217	113
39 hr		1217	390
40 hr		1217	113
41 hr		1217	390
42 hr		1217	113
43 hr		1217	390
44 hr		1217	113
45 hr		1217	390
46 hr		1217	113
47 hr		1217	390
48 hr		1217	113
49 hr		1217	390
50 hr		1217	113
51 hr		1217	390
52 hr		1217	113
53 hr		1217	390
54 hr		1217	113
55 hr		1217	390
56 hr		1217	113
57 hr		1217	390
58 hr		1217	113
59 hr		1217	390
60 hr		1217	113
61 hr		1217	390
62 hr		1217	113
63 hr		1217	390
64 hr		1217	113
65 hr		1217	390
66 hr		1217	113
67 hr		1217	390
68 hr		1217	113
69 hr		1217	390
70 hr		1217	113
71 hr		1217	390
72 hr		1217	113
73 hr		1217	390
74 hr		1217	113
75 hr		1217	390
76 hr		1217	113
77 hr		1217	390
78 hr		1217	113
79 hr		1217	390
80 hr		1217	113
81 hr		1217	390
82 hr		1217	113
83 hr		1217	390
84 hr		1217	113
85 hr		1217	390
86 hr		1217	113
87 hr		1217	390
88 hr		1217	113
89 hr		1217	390
90 hr		1217	113
91 hr		1217	390
92 hr		1217	113
93 hr		1217	390
94 hr		1217	113
95 hr		1217	390
96 hr		1217	113
97 hr		1217	390
98 hr		1217	113
99 hr		1217	390
100 hr		1217	113

Experiment #10

Fred D. Amoani #263 Age 46 Ht 5'8" Wt 145 lbs

First dosing formulation - Hoffmann-La Roche
Dosed on 11/31/83 1983

Time Post Dose	Radioactive Time	Count (CPM)
-15 min	1710	10
0 time	1805	10
1 hr	1815	100
1 hr	1805	190
4 hr	1803	113
5 hr	1800	53
8 hr	1800	111
10 hr	1800	148
11 hr	1800	100
12 hr	1800	10
13 hr	1800	10000
15 hr	1800	100
16 hr	1800	535
17 hr	1800	543
18 hr	1800	574
19 hr	1800	593
20 hr	1800	593
21 hr	1800	148
22 hr	1800	507
23 hr	1800	10000
24 hr	1800	100
25 hr	1800	100
26 hr	1800	100
27 hr	1800	100
28 hr	1800	100
29 hr	1800	100
30 hr	1800	100
31 hr	1800	100
32 hr	1800	100
33 hr	1800	100
34 hr	1800	100
35 hr	1800	100
36 hr	1800	100
37 hr	1800	100
38 hr	1800	100
39 hr	1800	100
40 hr	1800	100
41 hr	1800	100
42 hr	1800	100
43 hr	1800	100
44 hr	1800	100
45 hr	1800	100
46 hr	1800	100
47 hr	1800	100
48 hr	1800	100
49 hr	1800	100
50 hr	1800	100
51 hr	1800	100
52 hr	1800	100
53 hr	1800	100
54 hr	1800	100
55 hr	1800	100
56 hr	1800	100
57 hr	1800	100
58 hr	1800	100
59 hr	1800	100
60 hr	1800	100
61 hr	1800	100
62 hr	1800	100
63 hr	1800	100
64 hr	1800	100
65 hr	1800	100
66 hr	1800	100
67 hr	1800	100
68 hr	1800	100
69 hr	1800	100
70 hr	1800	100
71 hr	1800	100
72 hr	1800	100
73 hr	1800	100
74 hr	1800	100
75 hr	1800	100
76 hr	1800	100
77 hr	1800	100
78 hr	1800	100
79 hr	1800	100
80 hr	1800	100
81 hr	1800	100
82 hr	1800	100
83 hr	1800	100
84 hr	1800	100
85 hr	1800	100
86 hr	1800	100
87 hr	1800	100
88 hr	1800	100
89 hr	1800	100
90 hr	1800	100
91 hr	1800	100
92 hr	1800	100
93 hr	1800	100
94 hr	1800	100
95 hr	1800	100
96 hr	1800	100
97 hr	1800	100
98 hr	1800	100
99 hr	1800	100
100 hr	1800	100

FILMED
58